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Review

Yeast and filamentous fungi as model organisms in microbody research

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Abstract

Yeast and filamentous fungi are important model organisms in microbody research. The value of these organisms as models for higher eukaryotes is underscored by the observation that the principles of various aspects of microbody biology are strongly conserved from lower to higher eukaryotes. This has allowed to resolve various peroxisome-related functions, including peroxisome biogenesis disorders in man. This paper summarizes the major advances in microbody research using fungal systems and specifies specific properties and advantages/disadvantages of the major model organisms currently in use.

Keywords: Peroxisome; Glyoxysome; Hydrogenosome; Yeast; *PEX* gene; Pexophagy; Woronin body

1. General introduction

Lower eukaryotes, like yeast and filamentous fungi, are attractive organisms to study fundamental processes of the eukaryotic cell. Relative to higher eukaryotes, yeast have the advantage of easy cultivation on simple defined media with short generation times and easy accessibility towards molecular and classical genetics. The entire genome sequence of a growing number of yeast and fungal species is available, which enables to extensively apply modern system biology approaches. The fact that fungi are more related to animals than to plants emphasizes the value of these organisms as favourable models for human cells.

During the last decades, different yeast species have been used extensively as model organisms to study the function, biogenesis and maintenance of microbodies (peroxisomes, glyoxysomes, hydrogenosomes) [1]. Microbodies are cell organelles that have a very simple morphology as they consist of a proteinaceous matrix that is surrounded by a single membrane [2].

In yeast, microbodies are predominantly involved in the primary metabolism of various unusual carbon- and organic nitrogen sources used for growth [1].

A major advantage of using yeast in microbody research is the observation that microbody malfunctioning or deficiency is not lethal. This property has allowed isolating mutants in which peroxisome biogenesis was affected (termed *pex* mutants) and to clone the corresponding genes (*PEX* genes) by functional complementation [3]. At present 32 different *PEX* genes are known, most of which are conserved among higher and lower eukaryotes. Their analysis has led to a detailed understanding of various aspects of peroxisome formation and stability. Because of the conservation of the principles of peroxisome biogenesis, the knowledge obtained from yeast research has greatly contributed to the elucidation of the molecular basis of several inherited human peroxisomal diseases, e.g., Zellweger syndrome [3].

In filamentous fungi microbodies also play a role in the primary carbon metabolism, but in addition they can perform biosynthetic and structural functions. In *Penicillium chrysogenum* the final steps of the biosynthetic pathway of the antibiotic penicillin are catalyzed by microbody enzymes [4,5], whereas in ascomycetous fungi septal pores can be sealed by a highly specialized microbody, the Woronin body [6]. In anaerobic fungi hydrogenosomes exist, which play an important role in anaerobic growth [7,8].

In this contribution we present an overview of yeast and filamentous fungi, which are frequently used in microbody

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research. Emphasis is placed on major advancements as well as specific properties of the various model systems.

2. Yeast

2.1. Methylotrophic yeast

2.1.1. Methanol-induced peroxisome proliferation

From all organisms analyzed so far, the highest peroxisome abundance has been encountered in methylotrophic yeast species (e.g., *Candida boidinii*, *Hansenula polymorpha*, *Pichia pastoris*). In these organisms peroxisomes massively develop during growth of cells on methanol as sole source of carbon and energy [1,9,10]. This phenomenon has already been described in 1975, when research on peroxisomes was still in its infancy because at that time the significance of the organelles in cellular metabolism was not considered very important [11,12].

In methanol-grown yeast cells peroxisomes harbor three key enzymes of methanol metabolism, alcohol oxidase (AO), dihydroxyacetone synthase (DHAS) and catalase (CAT). The abundance of peroxisomes in these cells is related to the very high protein levels of AO and DHAS (up to approximately 70% of the total cellular protein). The high intra-organellar AO concentration results in crystallization of this protein, which is reflected in the typical cuboid shape of the organelles [13]. Where methanol-grown cells are crowded with large peroxisomes, the situation in glucose-grown cells is completely the opposite because the synthesis of AO and DHAS is fully repressed. As a consequence only one – or infrequently very few – small peroxisomes are present per cell (Fig. 1). Transfer of glucose-grown cells to methanol media results in a rapid and extreme make-over of the subcellular structure of the cells, which renders these organisms very attractive to study the process of peroxisome proliferation [14,15] (Fig. 1).

Detailed morphological studies in *H. polymorpha* indicated that upon transfer of glucose-grown cells to methanol medium, initially the single small peroxisome increased in size due to incorporation of newly synthesized peroxisomal proteins and membrane lipids. Growth of this organelle ceased when it has reached a defined, mature size. At this point one – or infrequently few – new organelles are formed, which subsequently start to grow. Hence, within one cell different types of peroxisomes exist with respect to their capacity to grow [16]. Other modes of peroxisomes proliferation have also been observed. In *C. boidinii* the few small organelles present in the glucose-grown cells initially rapidly proliferated followed by growth of the individual organelles. However, also in *C. boidinii* peroxisomes are only temporarily capable to import peroxisomal matrix proteins [17]. Differential import of matrix proteins in distinct sub-populations of peroxisomes has also been described for the non-methylotrophic yeast *Y. lipolytica* [18] and the filamentous fungus *Neurospora crassa* [19], but has not been demonstrated in higher eukaryotes.

The opposite process of peroxisome proliferation, namely the rapid removal of bulk of the organelles, can also simply be manipulated in methylotrophic yeast by changing the composition of the growth medium. Upon exposure of methanol-grown cells to excess glucose or ethanol, the organelles harboring key enzymes of methanol metabolism become redundant for growth and are rapidly and selectively degraded by autophagy (also designated pexophagy). This phenomenon was already described for *C. boidinii* and *H. polymorpha* in the seventies of the last century [20,21].

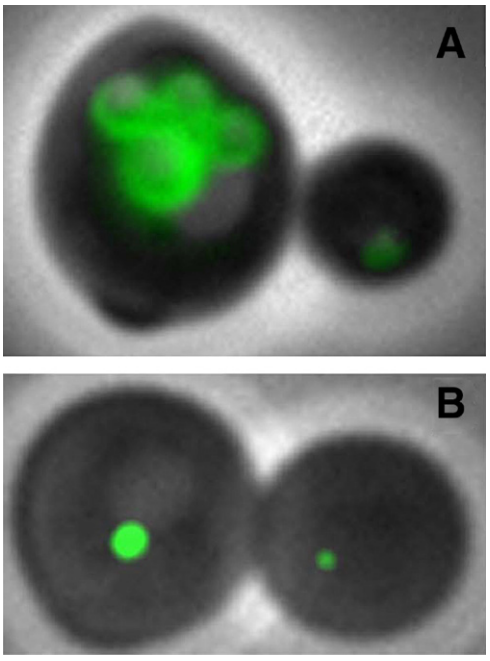

Fig. 1. Peroxisome proliferation in the methylotrophic yeast *Hansenula polymorpha*. Overlays of bright field and fluorescence images of methanol (A)- and glucose (B)-grown cells producing green fluorescent protein that contains a PTS1 (GFP-PTS1). During growth on glucose generally only one, small peroxisome is present per cell, whereas during methanol growth both the number and size of the organelles strongly increase.

Two different modes of pexophagy occur in yeast, namely micro- and macropexophagy. These processes share morphological characteristics with micro-and macroautophagy respectively. In micropexophagy vacuoles form protrusions that enclose and finally engulf clusters of peroxisomes. In macropexophagy individual peroxisomes are first sequestered from the cytoplasm by 2 or more membrane layers to form an autophagosome. Subsequently, the outer membrane of the autophagosome fuses with the vacuolar membrane, resulting in the release of the enclosed organelle in the vacuole and its subsequent degradation [22,23].

Studies in *H. polymorpha* suggested that in particular mature peroxisomes are degraded leaving the nascent organelles unaffected. The protection of these organelles against degradation most likely has the major physiological advantage that it allows the cell to rapidly adapt to new growth environments, which require new peroxisomal enzymes. These observations also indicate that a relationship exists between matrix protein import competence and sensitivity towards pexophagy. In line with this assumption are the findings that in *H. polymorpha* Pex3p and Pex14p, peroxins required for organelle development, are also playing a role in pexophagy [24–26].

Using plate assays which allow to detect AO enzyme activity, *H. polymorpha* and *P. pastoris* mutants defective in selective peroxisome degradation have been isolated [27,28]. This opened the way to clone the corresponding genes by functional complementation [28,29]. Interestingly many of these genes were already known from other cellular processes. For instance, the first *H. polymorpha* protein that was shown to be required for pexophagy was the homologue of *Saccharomyces cerevisiae* Vps34, a phosphatidylinositol 3-kinase involved in vacuolar protein sorting [30]. The first *P. pastoris* pexophagy gene is *PFK1*, a gene encoding the regulatory subunit of phosphofructokinase. Remarkably, the role of this protein in pexophagy was independent of its enzyme activity [29]. Another *P. pastoris* gene required for pexophagy is homologous to the *S. cerevisiae APG7/CVT2* gene [31]. This gene was initially identified to play a role in the cytoplasm to vacuole targeting (Cvt) pathway and macroautophagy in *S. cerevisiae*. Now several additional examples are known of *S. cerevisiae* genes that are required for pexophagy, but also play a role in the Cvt and autophagy pathways. Apparently the components that are involved in these three pathways for delivery of cellular components to the vacuole partially overlap. Because of the range of names that was used to indicate these genes (e.g. *APG*, *CVT*, *GSA*, *PDD*) a unified nomenclature has been adopted to prevent confusion, namely *ATG*, which stands for 'AuTophaGy-related' [32].

2.1.2. Induction of peroxisome proliferation by ethanol, acetate and oleic acid

During growth of methylotrophic yeast cells on ethanol or acetate glyoxysomes are formed, which harbor the glyoxylate cycle enzymes, isocitrate lyase and malate synthase. Growth on ethanol or acetate results in slightly enhanced peroxisome abundance relative to glucose-grown cells [1].

In *C. boidinii* and *P. pastoris*, but not in *H. polymorpha*, peroxisome proliferation is also induced during growth of cells on oleic acid. These peroxisomes harbor the enzymes of the beta-oxidation pathway. Incubation of *H. polymorpha* cells in oleic acid media results in the induction of enzymes of the beta-oxidation pathway and excessive proliferation of peroxisomal membranes [33]. It is still unknown why these membranes are formed and why *H. polymorpha* fails to utilize this carbon source.

2.1.3. Metabolism of organic nitrogen sources by peroxisomal oxidases

Methylotrophic yeast species are capable to utilize several of organic nitrogen sources, which are initially metabolized by peroxisome-borne oxidases. Synthesis of these enzymes is generally repressed by ammonium. Examples are amine oxidase (for utilization of primary amines like methylamine or ethylamine), urate oxidase and D-amino acid oxidase (e.g., for the utilization of D-alanine). Together with these oxidases also peroxisomal catalase is induced, paralleled by a slight increase in peroxisome size and number [14].

Interestingly, *C. boidinii* is capable to utilize D-alanine as the combined carbon and nitrogen source [34,35]. Hence, in these cells all carbon and nitrogen required for growth flows through peroxisomes, accompanied by relatively strong peroxisome proliferation. A similar phenomenon occurs in certain non-methylotrophic yeast species, which are capable to grow on primary amines or purines as sole source of nitrogen, carbon and energy (e.g., *Trichosporon cutaneum* [36]).

2.1.4. Peroxisome biogenesis in methylotrophic yeast

Almost all peroxisomal enzymes in methylotrophic yeast are imported via the PTS1 protein import pathway. Exceptions are *P. pastoris* thiolase and *H. polymorpha* amine oxidase, which are PTS2 enzymes.

To date a large number of *H. polymorpha* and *P. pastoris* *PEX* genes are known. From the first series of 22 published *PEX* genes all *H. polymorpha* and *P. pastoris* homologues have been identified, except for *PEX9*, *PEX11*, *PEX15*, *PEX16*, *PEX18* and *PEX21* (for a recent overview of yeast and fungal *PEX* genes see [37]). *PEX18* and *PEX21* have so far only been identified in *S. cerevisiae*, where these genes are required for import of PTS2 proteins [38]. In methylotrophic yeast the function of these genes is fulfilled by a single gene, *PEX20* [39,40].

For *C. boidinii* only *PEX11* has been described [41]. Genetic studies on *C. boidinii* are generally difficult because this yeast lacks a sexual cycle and most laboratory strains are diploids. Transformation and mutagenesis procedures, however, can be applied in an available haploid *C. boidinii* laboratory strain [42–44].

2.1.5. Application of methylotrophic yeast species

The methylotrophic yeast species *H. polymorpha*, *C. boidinii* and *P. pastoris* have found wide applications as attractive cell factories. These organisms have the advantage of the availability of various strong promoters (e.g., the alcohol oxidase promoter) to drive heterologous gene expression and are now being used for industrial production of various valuable pharmaceutical proteins, e.g., a hepatitis B antigen [45]. Besides this, *H. polymorpha* is specifically attractive for the heterologous production of membrane proteins. This option is stressed by the observation that various inherited diseases are related to membrane protein malfunction. Hence, a reliable system to overproduce these proteins is of great medical interest, e.g., for rational drug design. These proteins can be sorted to an ideal niche, namely the relatively "empty" peroxisomal membranes that do not or hardly contain homologous proteins and are massively proliferate during incubation of cells on oleate [33].

Moreover, methylotrophic yeast species have the advantage that they are crowded with "enzyme bags" (peroxisomes) that contain the key enzymes of methanol metabolism. This has opened the way to replace the protein contents of these bags and use them as storage compartment of heterologous compounds that are unstable or toxic for the cell at normal intracellular production. To this end, the heterologous gene is fused to a PTS1 signal to allow uptake in peroxisomes. If required, a TEV protease cleavage site can be introduced in front of the PTS1. At simultaneous production of the TEV protease in peroxisomes the PTS1 signal can readily be removed from the protein inside

the organelles. This is not harmful for the cells, as TEV producing *H. polymorpha* cells grow normally on defined media [46]. A successful example of this peroxisome-based strategy is the high-level production of fungal fructosyl amino acid oxidase in peroxisomes of *C. boidinii* [47].

2.2. Saccharomyces cerevisiae

2.2.1. Oleic acid-induced peroxisome proliferation

S. cerevisiae peroxisomes were for the first time morphologically described by Avers and Federman in 1968 [48] and isolated from cell homogenates by Parish in 1975 [49]. It took however another 12 years before collaborative efforts of the groups of Veenhuis and Kunau resulted in the finding that during growth on oleic acid peroxisome proliferation is induced in *S. cerevisiae* [50]. Still, the organelle abundance in these cells is relatively low as compared to that observed in methanol-grown methylotrophic yeast.

Growth of *S. cerevisiae* cells on ethanol did not result in strongly enhanced peroxisome proliferation relative to glucose-grown cells. Also, none of the organic nitrogen source tested resulted in enhanced peroxisome proliferation [50].

The finding how to induce peroxisomes in bakers yeast was the onset of a new era in peroxisome research, which among others resulted in the isolation of the first *S. cerevisiae pex* mutant and *PEX* gene in 1989 and 1991, respectively [51,52]. At present 25 *S. cerevisiae PEX* genes have been characterized. Of the first group of 22 *PEX* genes, all *S. cerevisiae* homologues have been identified, except for *PEX9*, *PEX16* and *PEX20* [37].

2.2.2. Pexophagy in S. cerevisiae

In *S. cerevisiae* pexophagy is induced by adding glucose to oleic acid-grown cells [53,54] or transferring these cells to glucose rich, nitrogen limited media [55]. A large number of genes previously identified in autophagy or the Cvt pathway were demonstrated to be also required for pexophagy (for a review see [56]). Specific screening procedures for the isolation of *S. cerevisiae* mutants defective in pexophagy have not been reported yet. However, a wealth of mutants affected in autophagy or the Cvt pathway are available, which all are likely candidates to be blocked in pexophagy as well [56].

2.2.3. Systems biology in S. cerevisiae peroxisome research

Peroxisome research in *S. cerevisiae* has taken strong advantage of the availability of a wealth of classical and molecular genetic tools and expertise. In addition, the *S. cerevisiae* genome was the first eukaryotic genome that was sequenced. This opened the way to identify novel peroxisomal matrix proteins by data base searches using typical peroxisomal characteristics, like PTS sequences or oleate response elements (OREs) [57]. Another spin-off of this milestone is the current availability of large collections of highly valuable *S. cerevisiae* strains, like deletion strains of most non-essential *S. cerevisiae* genes. Moreover, large yeast two-hybrid databases, protein interaction maps and protein localization databases are available, which brought systems biology into *S. cerevisiae* peroxisome research.

Initial studies included Serial Analysis of Gene Expression [58] to compare mRNA levels in glucose- and oleate-grown *S. cerevisiae* cells. As anticipated the levels of mRNAs encoding peroxisomal enzymes of the β-oxidation pathway were significantly increased in oleate-grown cells. However, with the exception of Pex11p, the mRNA levels of *PEX* genes were rather low at both growth conditions. As a consequence, novel peroxins were not identified by this method.

Later transcriptome studies using DNA microarrays [59] resulted in the identification of *PEX25*, which like *PEX11* plays a role in regulating organelle abundance. However, also transcriptome profiling studies have not uncovered novel *PEX* genes yet that play a direct role in peroxisome formation.

Fluorescence microscopy of the extensive collection of *S. cerevisiae* strains that produce GFP fusion proteins has led to identification of novel peroxisomal proteins of unknown function [60]. One of these, Inp1p, turned out to fulfill a novel peroxisome function, namely in regulating the proper partitioning of peroxisomes over mother cell and bud during yeast budding. Most likely Inp1p, which is a peroxisomal membrane protein, links peroxisomes to cortical anchors in mother cells and buds [61]. In a similar way Inp2p was identified, a peroxisome-specific receptor for Myo2p [62].

Also organelle proteomics was introduced in *S. cerevisiae* peroxisome research. Several laboratories have performed the laborious and difficult job to isolate highly pure peroxisome fractions and to analyze their protein content. However, these studies were severely hampered by contaminating proteins from other cell compartments in peroxisomal fractions [63–65]. However, recently Marelli and colleagues [66] reported on quantitative mass spectrometry using isotope-coded affinity tags (ICAT) and a novel scoring algorithm to determine whether proteins were specifically co-enriched with peroxisomal marker proteins or not. These analyses led to the identification of several proteins that were already known to function at other subcellular sites, but which apparently have a dual location and are also present at peroxisomes. Examples of such proteins include Vps1, Rho1p and Emp24p. In yeast, Rho1p regulates polarized growth by organizing cortical actin patches at defined positions in the cell. Apparently, the surface of peroxisomes is one of these positions, where it was suggested to be required for peroxisome fission [66].

Emp24p is a member of the p24 protein family. These proteins are components of coated (COP) vesicles, which mediate intracellular trafficking between the ER and Golgi apparatus [67]. Recent studies in *H. polymorpha* suggested that Emp24p and other members of the p24 protein family are required for the formation of new peroxisomes from the ER (i.e., in yeast cells lacking pre-existing peroxisomes). The absence of p24 proteins in a *H. polymorpha* WT background resulted in a very prominent decrease in organelle number in conjunction with a strong increase in the size of the individual organelles [68]. Possibly, COP vesicles deliver peroxisomal membrane components to pre-existing peroxisomes. In the absence of p24 proteins the efficiency of this pathway may be

reduced leading to less but larger organelles, as these have a lower surface to volume ratio.

2.3. Yarrowia lipolytica

2.3.1. Peroxisome biogenesis

Y. lipolytica cells grow very well on oleate and n-alkanes, but not on methanol. Growth on oleate is accompanied by strong induction of peroxisomes. *Y. lipolytica* is also capable to utilize ethylamine – but not D-amino acids or uric acid – as sole source of nitrogen, which is metabolized by a peroxisomal amine oxidase [69].

The first *Y. lipolytica pex* mutant and *PEX* gene were isolated in 1993–1994 [70,71]. To date most of the common yeast *PEX* genes are also described for *Y. lipolytica* [37]. So far, a *PEX9* gene was only identified in *Y. lipolytica* [72]. Reinspection of the putative *Y. lipolytica PEX9* sequence, however, recently suggested that the *Y. lipolytica PEX9* gene in fact encodes a Pex26p ortholog [37].

A remarkable *Y. lipolytica PEX* gene is *PEX16*, because this gene was so far only identified in higher eukaryotes (plant and mammalian cells) [72]. *PEX16*, together with *PEX3* and *PEX19*, has been implicated in the formation of peroxisomal membranes. In the absence of these genes peroxisomal membrane ghosts are generally absent and peroxisomal membrane proteins are instable or mislocalized.

It has been proposed that Pex3p and Pex19p play key roles in recognition and insertion of newly synthesized peroxisomal membrane proteins in peroxisomal membranes (reviewed in [73]). The role of Pex16p is still speculative, but it has been suggested to function upstream of Pex3p and Pex19p [74].

Interestingly, data have been presented that show that YlPex16p traffics via the ER to peroxisomes. In pulse labeling experiments YlPex16p, produced at normal levels, was shown to be initially targeted from the cytosol to the ER, where it was N-glycosylated in the ER lumen, and subsequently chased to peroxisomes [75].

This process was blocked or strongly retarded in *Y. lipolytica* mutants carrying mutations in genes involved in protein secretion (*SEC238* and *SRP54*) or peroxisome biogenesis (*PEX1* and *PEX6*). These data suggest that Pex1p and Pex6p may play an important role in exit of Pex16p from the ER in addition to their function in recycling of Pex5p at the peroxisomal membrane [76].

Recent studies in *Arabidopsis* indicated that plant Pex16 has a dual location in the ER and peroxisomes [77], which may also point to an ER to peroxisome pathway of Pex16p in plant. Possibly this process involves p24 proteins and COP vesicles.

2.3.2. Pexophagy in Y. lipolytica

In *Y. lipolytica* pexophagy can be induced upon exposure of acetate/oleate-grown cells to glucose excess conditions. Electron microscopic analysis failed to uncover the formation of autophagosomes, and hence it was concluded that the organelles are most likely degraded by micropexophagy [69]. However, recent fluorescence microscopy studies suggested a macropexophagy process, because changes in the curvature of the vacuolar membranes were not detected at the site of peroxisome uptake [78].

A plate assay screening procedure has been developed to isolate peroxisome degradation-defective mutants and the isolation of such mutants has been reported [69]. However, so far it is unknown which genes were affected in these mutants.

3. Filamentous fungi

3.1. General remarks

In filamentous fungi four types of microbodies have been described. These include glyoxysomes, peroxisomes, Woronin bodies and hydrogenosomes. The latter organelles only occur in anaerobic fungi, which are abundantly present in the gastrointestinal tract of many herbivores where they participate in the degradation of plant polymers. Below the fungal microbodies are discussed in more detail.

3.2. Glyoxysomes, peroxisomes

Peroxisomes in fungi have been first characterized cytochemically by Reiss in 1971 [79]. Among filamentous fungi, *N. crassa* has been studied in more detail with respect to microbody biogenesis. This organism contains glyoxysomes during growth of cells on C_2 compounds as ethanol and acetate. Glyoxysomes are characterized by the presence of the key enzymes of the glyoxylate cycle, malate synthase and isocitrate synthase. These enzymes are localized to microbodies of various fungi [80,81]. *N. crassa* cannot grow on oleate but peroxisomal β-oxidation enzymes are strongly induced when oleic acid is added to C_2-grown cells. The question whether *N. crassa* may contain two classes of functional microbodies (glyoxysomes and peroxisomes that contain catalase) is still not yet solved. Thieringer and Kunau [19] demonstrated that peroxisomal catalase does not colocalize with the glyoxylate cycle enzymes and therefore may be present in another type of microbodies, together with uricase. However, *N. crassa* β-oxidation enzymes localize to glyoxysomes [82]. In plant glyoxysomes may contain typical peroxisomal enzymes like catalase and even may transform into peroxisomes, a process that occurs during transition of cotyledons from germinating oily seed to the green stage [83]. It is therefore not yet understood why in *N. crassa* two types of organelles develop separately in the cells.

In addition to glyoxysomes and peroxisomes *N. crassa* also can form Woronin bodies (see below), which represent the third microbody type in this fungus.

The sequence of the *N. crassa* genome was completed in 2003 [84]. A search for *N. crassa PEX* genes revealed that from the first 22 *PEX* genes, the *N. crassa* genome contains all but *PEX9*, *PEX15*, *PEX17*, *PEX18*, *PEX21* and *PEX22* [37,85]. Compared to the *PEX* genes present in the yeast species described above, *N. crassa* seems to be most similar to *Y. lipolytica*, as it also contains a *PEX16* gene and requires *PEX20* for PTS2 import [37,85].

Apart from a role in primary carbon and nitrogen metabolism microbodies have also other important functions in filamentous fungi. First, they are important in both a-sexual (asco)spore formation [86] and in sexual spore formation [87]. The latter came from studies in *Podospora anserina*, which indicated that a mutation in a *PEX* gene (*PEX2*) caused a defect in karyogamy. Surprisingly, this defect could be suppressed by overexpression of PMP70, a peroxisomal ABC transporter [87].

A second important feature of fungal microbodies is that they can be involved in the production of secondary metabolites. An economically important example is their crucial role in the biosynthesis of β-lactam antibiotics (penicillins) in specific fungi (e.g., *P. chrysogenum* and *Aspergillus nidulans*), which crucially depends on peroxisome functions. The first enzyme of the penicillin biosynthetic pathway is the cytosolic non-ribosomal peptide synthetase ACV synthetase (ACVS) that assembles the three amino acids L-α-aminoadipate, L-cysteine and L-valine into the tripeptide δ-(L-aminadipyl)-L-cysteinyl-D-valine (ACV) [88]. After ring closure of ACV by isopenicillin N synthetase (IPNS), the resulting product, isopenicillin N (IPN), is translocated into the peroxisome for side chain exchange by isopenicillin N:acyl CoA acyltransferase (AT) to yield the final product [89]. Also the CoA ligase involved in side chain activation, is a peroxisome-borne enzyme [90]. This stresses the crucial function of the organelles in the synthesis of this industrially important compound.

Several *P. chrysogenum PEX* genes have been cloned and characterized (*PEX1*, *PEX5*, *PEX6* and *PEX11*[37,86,91,92]). Interestingly, overexpression of *PEX11* in *P. chrysogenum* resulted in massive peroxisome proliferation and a doubling of penicillin production [92] (Fig. 2).

3.3. Woronin bodies

Woronin bodies are present in ascomycetous fungi, where they generally function in response to cell damage and can seal septal pores to prevent leakage of cytoplasm. These structures develop as dense cores in specific, virtually normal microbodies, from which they subsequently separate to become independent structures. They generally assume a hexagonal shape which is caused by self-assembly of Hex1, its major protein constituent, into electron dense crystalline cores [6]. Such core structures are also formed when the *HEX1* gene is artificially expressed in *S. cerevisiae* [6]. *N. crassa* Hex1-deficient cells do not contain these high density cores and suffer from bleeding of cytoplasm through septa of damaged cells. Apparently, the dense core structure serves a specific function and is required to withstand the internal osmotic pressure of the cell in sealing the septal pore [93].

In a series of elegant experiments, Tey and colleagues [94] showed that in *N. crassa* Woronin body development is predominantly localized to the growing hyphal tip cells, a process that is prescribed in part by polarized expression of the *HEX1* gene. Intron splicing appeared to be essential for accumulation of *HEX1* transcripts. These results stress the use of filamentous fungi as attractive model systems to study localized gene expression and organelle assembly.

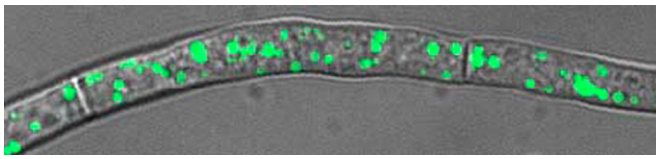

Fig. 2. Proliferation of peroxisomes in the filamentous fungus *Penicillium chrysogenum*. A hypha is shown of a *P. chrysogenum* strain that produces GFP-PTS1 to fluorescently mark peroxisomes (courtesy of DSM, The Netherlands). The picture represents an overlay of a bright field and fluorescence microscopy image obtained by confocal laser scanning microscopy.

Woronin bodies are also important for the pathogenicity of the rice blast fungus *Magnaporthe grise*. At the initial stage of infection, the Woronin body is required for the proper development and function of appressoria (infection structures). Upon infection, the Woronin body is necessary for survival of infectious fungal hyphae during invasive growth and host colonization [95]. These organelles contain a protein encoded by *MVP1*, which is homologous to *N. crassa* Hex1p [96].

3.4. Hydrogenosomes

Hydrogenosomes have been discovered approximately 35 years ago (for a review see [8]). These organelles are present in various unrelated anaerobic eukaryotes, which lack mitochondria (e.g., chytridiomycete fungi, anaerobic flagellates and ciliates). Generally, hydrogenosomes are spherical, 1–2 μm in diameter and lack DNA. Similar to microbodies, hydrogenosomal proteins are nuclear encoded, synthesized in the cytosol and translocated into the organelles. However, they may also have mitochondrial features [8].

Hydrogenosomes compartmentalize terminal reactions of anaerobic metabolism and produce hydrogen and ATP. Anaerobic chytrids (e.g., *Neocallimastix* and *Piromyces* species) metabolize malate (generated from glucose) to form ATP and acetate. The reduction equivalents that result from malate decarboxylation are not donated to a mitochondrial electron transport chain but instead used by a hydrogenase to form molecular hydrogen. However, under certain nutritional conditions, the import of pyruvate prevails, and hydrogen production becomes marginal, because pyruvate is metabolized by pyruvate: formate lyase without generation of reduction equivalents.

The molecular mechanisms of hydrogenosome biogenesis are still largely unknown predominantly because molecular techniques to study these organisms are scarce. Hydrogenosomes of different species vary in metabolic and structural properties [8].

Hydrogenosomes from the anaerobic fungus *Neocallimastix* are most likely bound by a single membrane, but contains large globular membranous inclusions [7]. It can however not be excluded that these inclusions have evolved from mitochondrial cristae. Nevertheless, these organelles seem to share mitochondria-related functions together with peroxisome characteristics. This is an excellent example of the plasticity of the organelle that allows it to adopt various functions from different cell compartments for own functions.

An exceptional type of hydrogenosomes is observed in *Nyctotherus ovalis* (an anaerobic ciliate that is present in the hindgut of cockroaches). Like mitochondria, these organelles have a double membrane and also contain a genome, which encodes components of a mitochondrial electron transport chain [97,98].

In an excellent study, the group of Hackstein presented evidence that *N. ovalis* organelles are of mitochondrial origin and obtained their hydrogenase activity via lateral gene transfer [98]. Analysis of a genomic DNA library of *N. ovalis* revealed the presence of nuclear genes that encode potential mitochondrial proteins including components of the mitochondrial import machinery. Hence, *N. ovalis* hydrogenosomes apparently are mitochondria that produce hydrogen. These organelles clearly represent a missing link between mitochondria and hydrogenosomes [98].

4. Concluding remarks

The data included in this paper demonstrate that the various yeast species and filamentous fungi used in modern microbody research all have their specific advantages and frequently complement each other to understand specific details. The predominantly used organism *S. cerevisiae* has of course the advantage of the availability of a wealth of tools and techniques. This for instance allowed to use for the first time system biology approaches that indeed uncovered novel processes related to peroxisomes (peroxisome inheritance, fission, organelle clustering etc.), but on the other hand did not result in the identification of novel peroxins that are directly involved in peroxisome formation (i.e., in protein sorting or membrane formation).

In classical screens for *pex* mutants bakers yeast may have the disadvantage of the occurrence of gene duplications, which may hamper the identification of novel genes because of redundancy effects. An example are the two peroxins Pex18p and Pex21p that are required for PTS2 protein import in *S. cerevisiae*, whereas in other yeast and filamentous fungi one protein fulfils this function (Pex20p).

Also the difference in peroxisome abundance between non-induced and induced cells is much smaller in *S. cerevisiae* than that observed in other yeast species. Hence, transcriptome profiling approaches may be more rewarding when applied on methylotrophic yeast species or *Y. lipolytica*.

An intriguing current question is whether all genes involved in peroxisome formation have now been identified. If not, the current data suggest that a continuation of classical random mutagenesis approaches in yeast may still be suitable to identify additional genes, whereas systems biology may uncover different classes of proteins involved in other processes in peroxisome biology.

Systems biology approaches predominantly resulted in identification of proteins in novel processes involved in peroxisome biology. The initial data showed that proteins can be involved in peroxisome biology that are already known to be required for other cellular processes (e.g., Emp24p, Rho1p, Vps1p). As some of these proteins are essential (e.g., Rho1p), it may be worthwhile to also isolate conditional yeast mutants defective in peroxisome biogenesis.

In pexophagy studies methylotrophic yeast species seem to be the organisms of choice, because of the ease of induction of these mechanisms. On the other hand, only for *S. cerevisiae* very large collections of mutants affected in autophagy, the Vps and Cvt pathways are available. As the process of pexophagy may strongly overlap with these pathways, these mutants are highly attractive for pexophagy research.

The use of various different yeast and fungal species remains very important to resolve which peroxins are the key players in peroxisome biology. For instance, Pex19p is a peroxin that has been proposed to represent a receptor for the targeting signals of peroxisomal membrane proteins (mPTSs [73]). However, in *Y. lipolytica* virtually normal peroxisomes can be formed in the absence of Pex19p [99]. Also, large peroxisomal structures are formed in *H. polymorpha pex19* cells that produce enhanced levels of Pex3p [68,100]. Hence, these data suggest that Pex19p cannot be the only, essential mPTS receptor in yeast.

Therefore, a broad approach, i.e., the use of various model organisms, remains to be important to resolve the general principles of microbody development and function.

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